FIG. 19. A definite wall separating the spore into two approximately equal parts.

FIG. 20. Same as preceding, but one part showing what may be interpreted as a generative nucleus and a tube nucleus.

III.

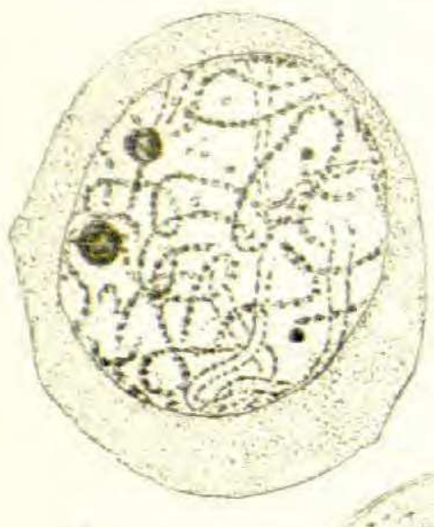
THE DIVISION OF THE MACROSPORE NUCLEUS.

JOHN H. SCHAFFNER.

(WITH PLATES XXXVII–XXXIX)

Although a knowledge of the changes which take place in the reduction nuclei of plants and animals is of the utmost importance, and will no doubt aid more than anything else in bringing about a correct interpretation of the facts of heredity, comparatively little has been done in this field, and the observations that have been reported disagree widely. This may be accounted for because of the extreme difficulty of properly preparing suitable material for study, and of correct observation and interpretation of the minute structures concerned. The following work was undertaken because especially favorable material was at hand, and some peculiar variations from what has been received as the normal process of reduction were observed. During the course of the investigation the writer was compelled several times to abandon preconceived notions obtained from the literature of the subject. Whatever, therefore, is presented in regard to the formation of chromosomes and the activities of the nucleoli during karyokinesis has not been the outcome of an attempt to establish evidence which would be agreeable to some hypothesis, but the whole investigation presented an array of facts conclusive to the writer's mind.

My thanks are due to Dr. John M. Coulter for his interest and supervision, as well as to a considerable number of coworkers in the laboratory who kindly permitted me to study and compare their preparations with my own.



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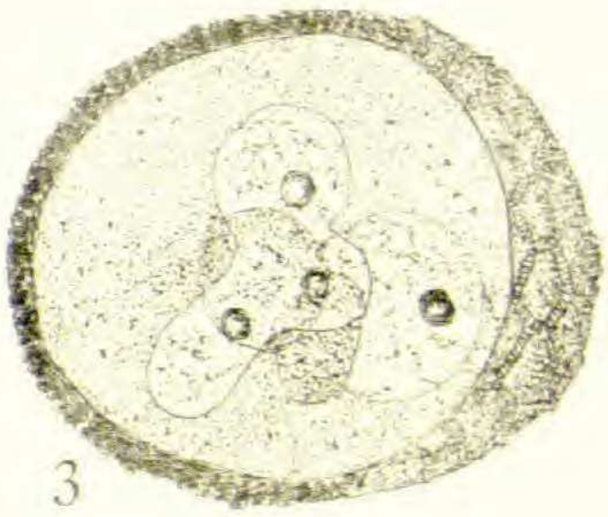

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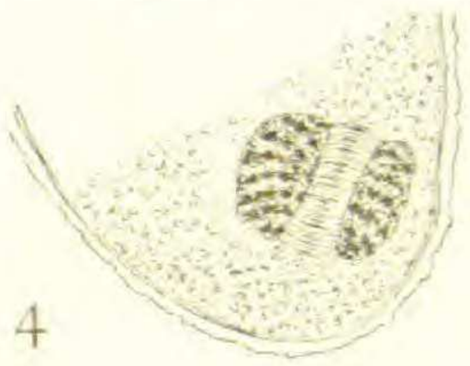

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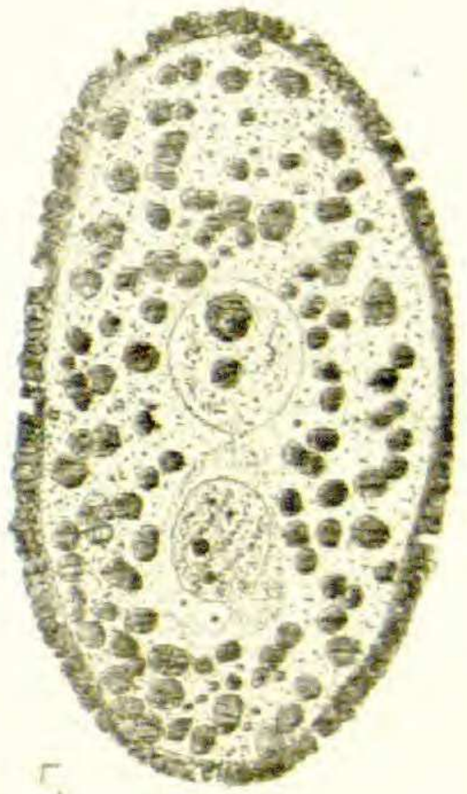

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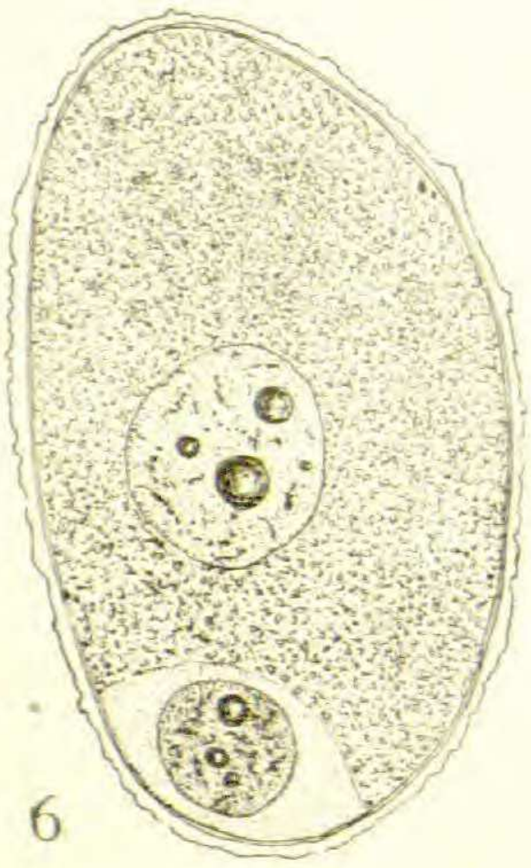

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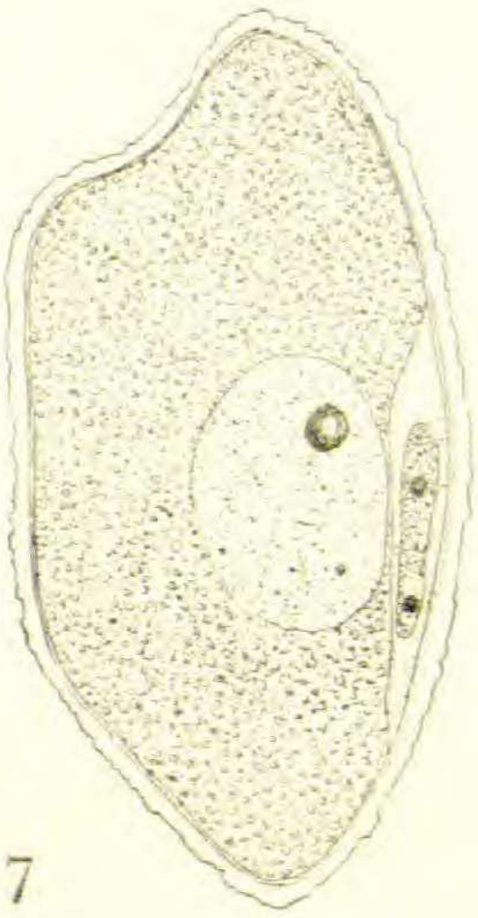

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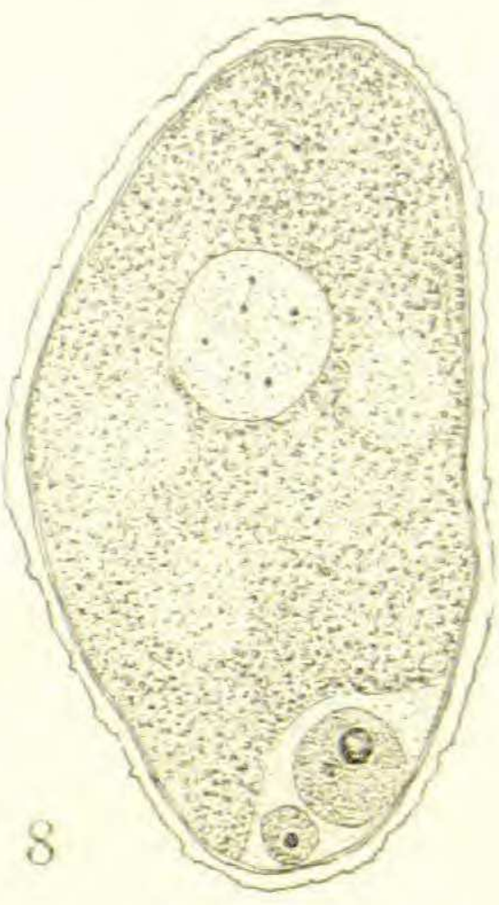

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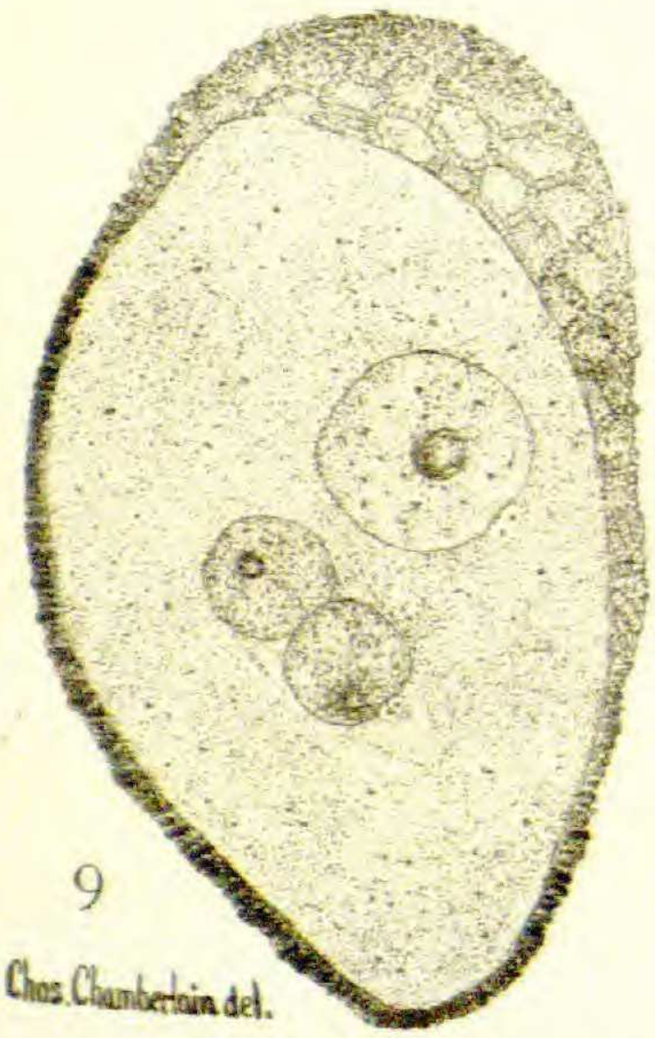

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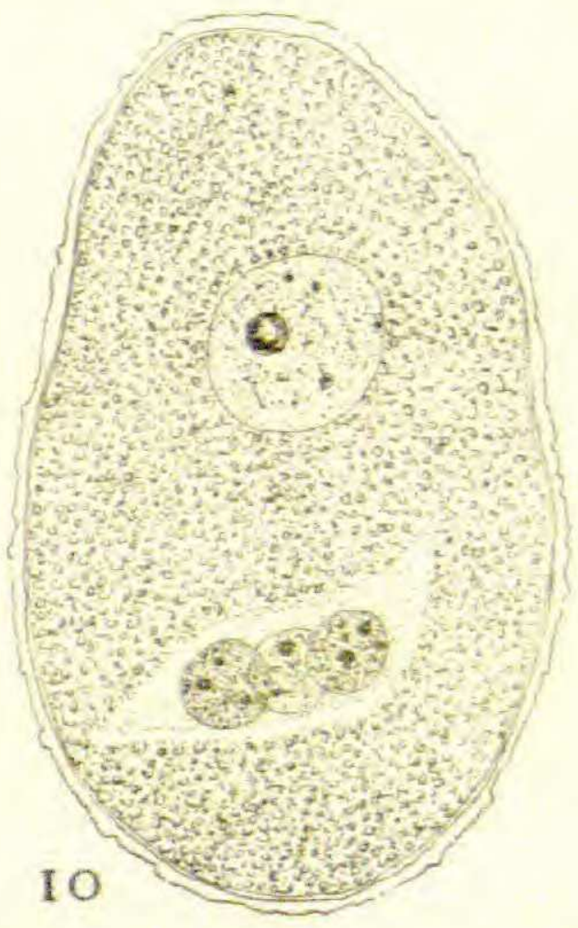

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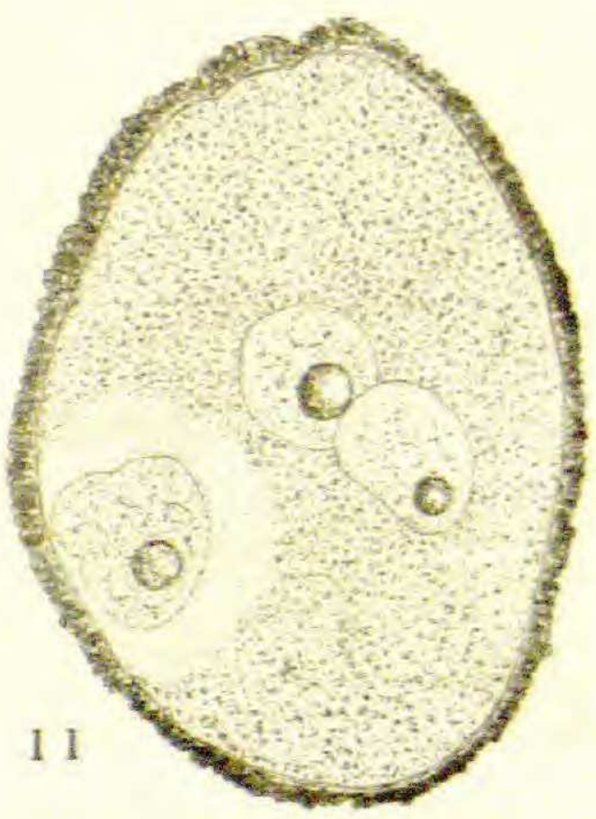

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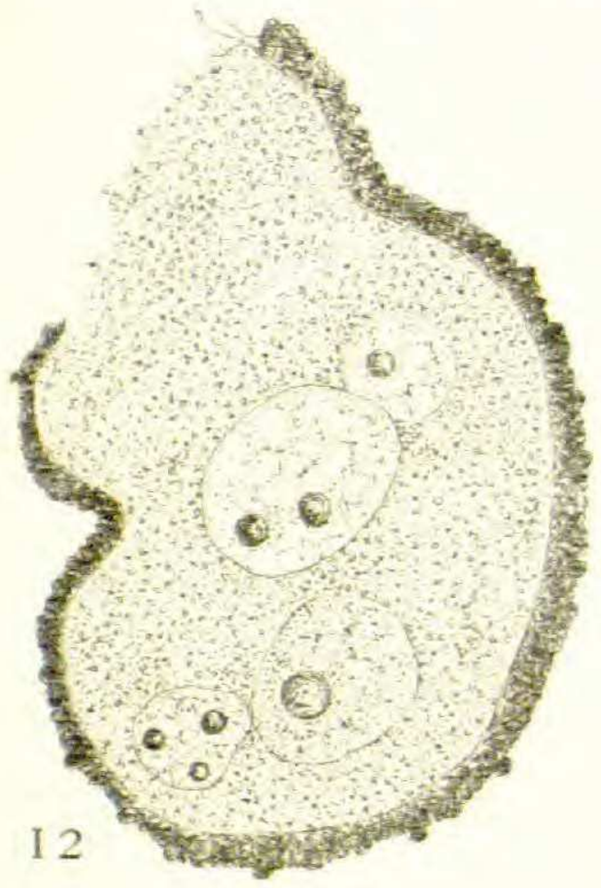
Chas. Chamberlain del.

CHAMBERLAIN on LILIUM.



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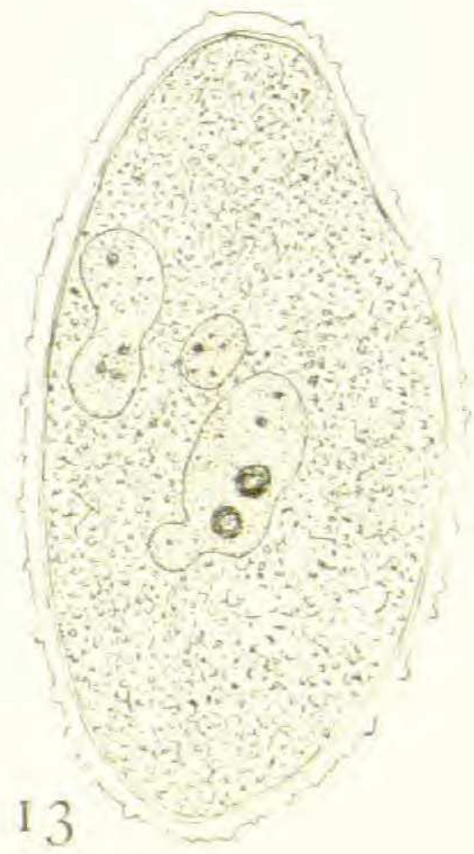

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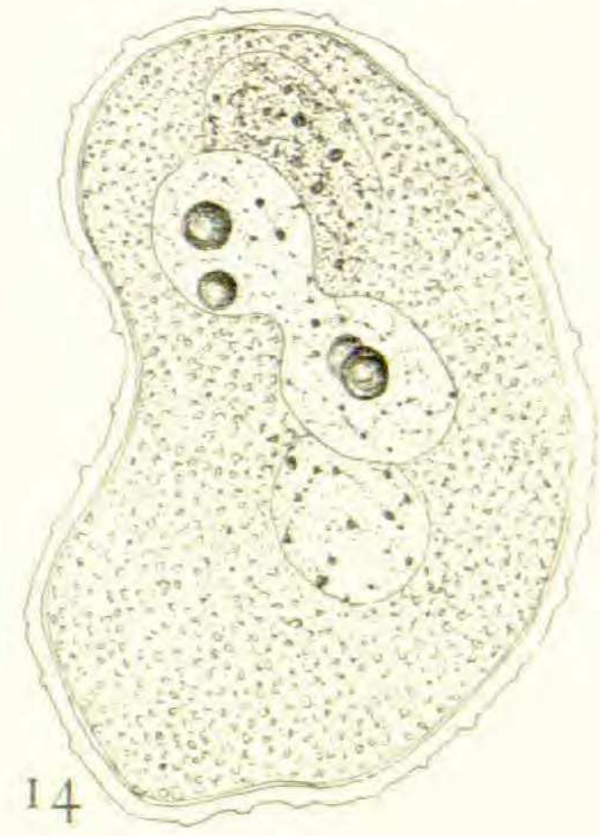

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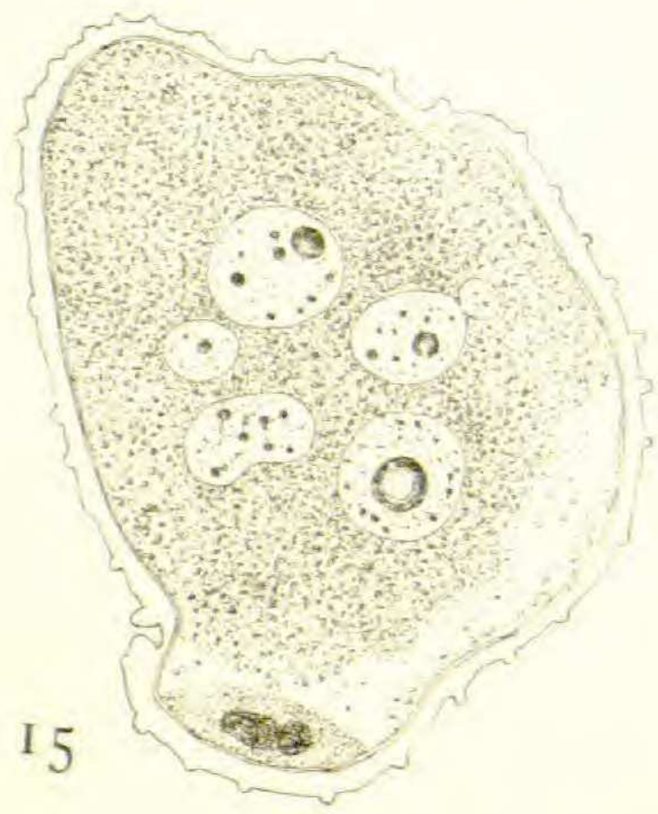

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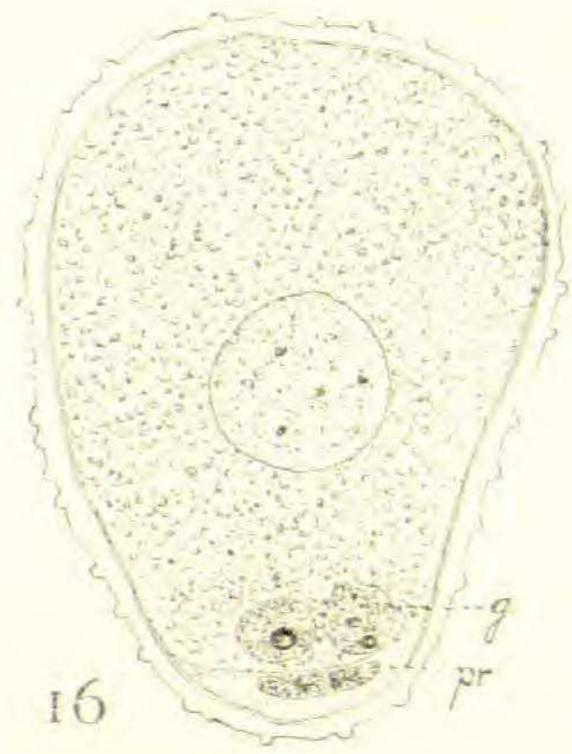

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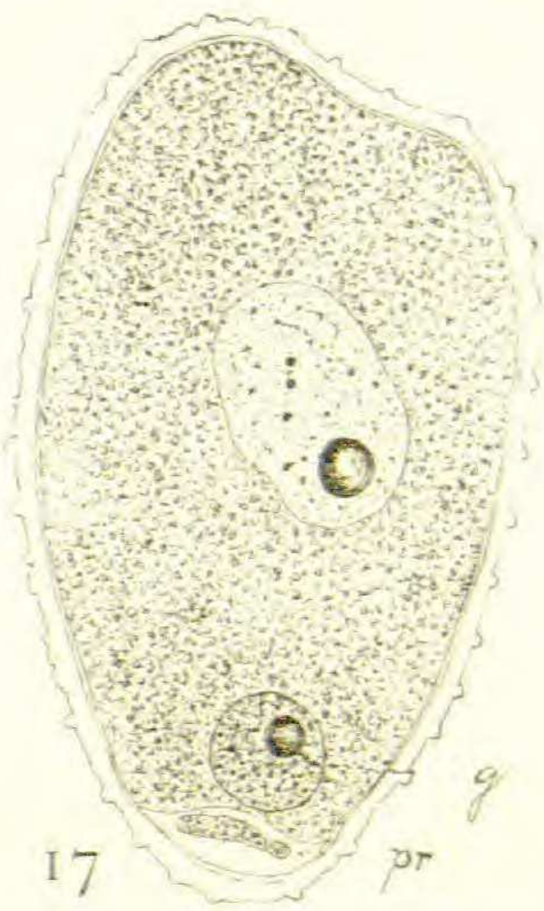

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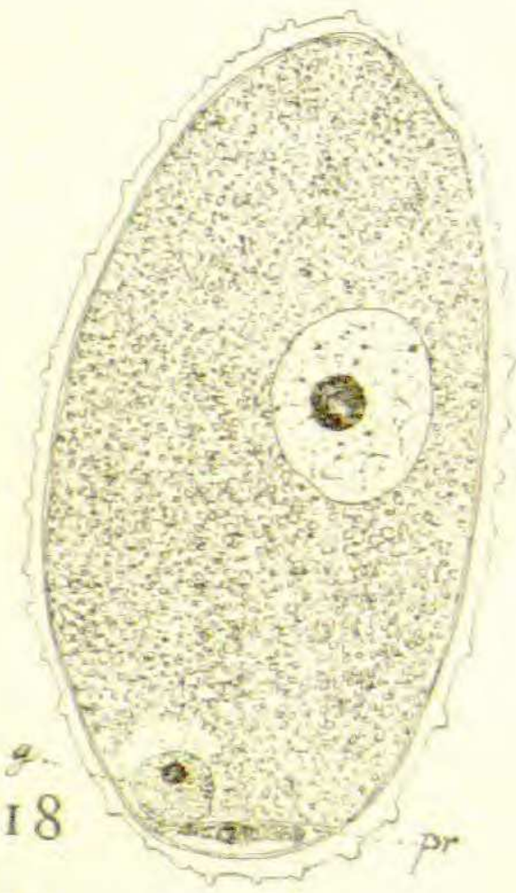

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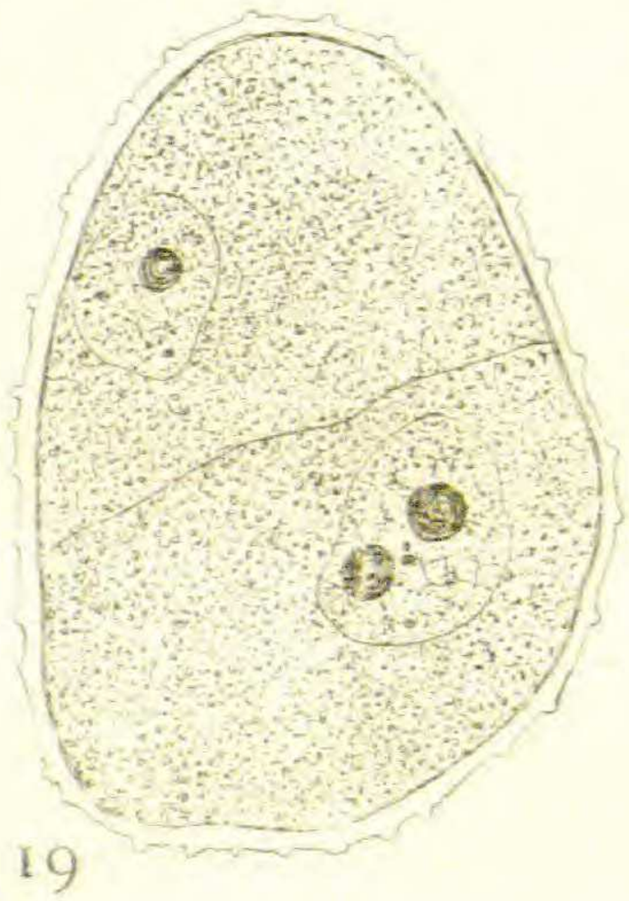

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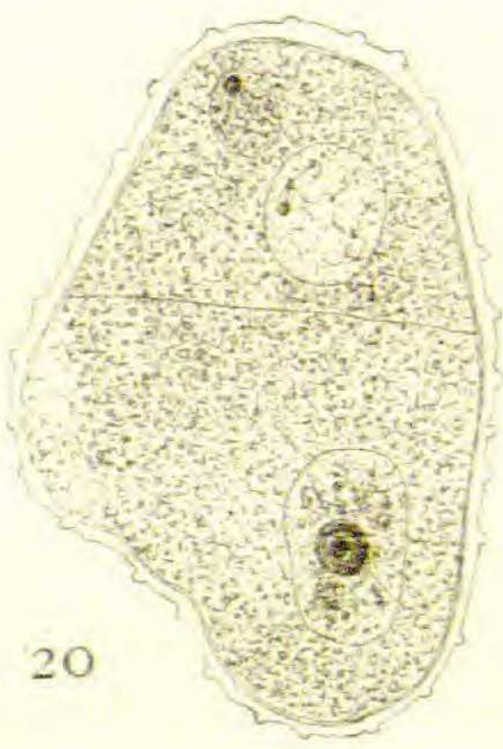

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Chas. Chamberlain del.

CHAMBERLAIN on LILIUM.

ACCOUNT OF INVESTIGATION.

In the young nucellus of *L. Philadelphicum* the archesporial cell soon shows its nature by a difference in size and staining reaction. As is well known, this cell in Lilium develops directly into the fertile macrospore, without cutting off a tapetal cell or dividing further into a number of macrospores. This gives an especially long period of growth for the development of the reduction nucleus. After the macrospore has attained some size its nucleus shows several large nucleoli, usually three in number, with the chromatin rather uniformly distributed and in close connection with the nucleoli. Whether the threads of the network really anastomose or not it would be difficult to determine, but such is the appearance. The threads contain numerous single chromatin granules which are arranged quite regularly, but are not all of the same size (*figs. 1, 1a*).

The chromatin network soon begins to thicken, and the granules grow larger, giving the nucleus a coarser appearance than in the earlier stages. The nucleoli at this stage usually have a homogeneous outer layer, while in the center is a large granular vacuole (*figs. 2, 2a, 3*). At the time when the integuments are just beginning to appear as minute projections on the side of the nucellus, the linin thread of the chromatin network becomes very thick and broad, and the chromatin granules undergo transverse divisions, making the whole network with double rows of chromatin granules instead of the former single row (*figs. 4, 4a*). At this stage, also, the whole chromatin band appears definitely to form a single continuous skein or spirem. At the same time, and even before, important changes are going on in the nucleoli. Sometimes these are of enormous size, with a great granular vacuole in the center. The nucleolus shown in *fig. 5* is larger than the average nucleus of the ovary tissue. In one case (*fig. 6*) such a nucleolus was found with a deep dent on one side. Whether or not this was caused by mechanical injury during preparation it is, of course, impossible to tell. The dent in this nucleolus may be of the same nature as the distortions which produce the so called "sickle stage," but here the

nucleolus was not in contact with the nuclear membrane, but was lying free in the nucleus. The "sickle stage," was seen only in poor preparations, and hence I am inclined to regard it as an artificial product. In many nucleoli at the same stage there are a number of smaller vacuoles, instead of a large central one (*fig. 7*). There is no doubt but that the term vacuole is a misnomer, but for lack of a better one this name will be used for the larger clearer areas in the nucleolus.

After the division of the chromatin granules the entire chromatin band or spirem undergoes longitudinal splitting, producing a double linin thread, each thread containing a single row of chromatin granules (*figs. 8–9a*). The double number of chromatin bands makes a very characteristic appearance when compared with the earlier stages before splitting. There does not appear to be any substance connecting the two chromatin bands, the longitudinal fission appearing complete.

At this stage there often appear peculiar radiations or tangential filaments in the cytoplasm. These generally stretch from one side of the cell to the other, passing the nucleus as tangents (*fig. 8*). Whether this appearance was an artificial production or not I could not determine, but it is probable that it is a natural condition, as the threads appeared in numerous sections which did not seem to be otherwise disturbed. At this stage the two centrospheres, which were sometimes seen, still lie close together beside the nucleus.

After the splitting of the chromatin band the two resulting bands now begin to twist on one another, the twisted spirem being in marked contrast with the former parallel arrangement (*figs. 10, 10a*). In the meantime the nucleus has enlarged considerably. After the two threads have twisted quite closely together the resulting twisted chromatin band arranges itself so as to form twelve loops, the heads of the loops being close to the nuclear membrane. Each loop contains from one to three twists. At first the double nature of the chromatin band is still very evident (*figs. 11-11b*), but later the two linin threads are much more intimately associated, almost giving the appearance

of a single ribbon with an irregular double row of chromatin granules (*figs. 11, 13*). At some point in this stage the so-called "synapsis" is said to occur. The chromatin loops now break apart and lie free in the nuclear area, while at the same time the nuclear membrane has almost entirely disappeared. Wherever the chromosomes were counted they were twelve in number. Thus it will be seen that the spirem first undergoes complete longitudinal fission and then breaks up into half the number of loops or chromosomes that are present in the cells of the sporophyte. The important feature in this pseudo-reduction of the number of chromosomes in the nucleus is not so much the fact that the spirem is cut into twelve parts as that it twists into twelve loops which predetermine the twelve divisions and the twelve chromosomes. The chromatin loops or chromosomes are not all of the same size. Indeed, there is often considerable difference in the lengths of the several chromosomes. In this way there may be considerable diversity in the subsequent reduction of the chromatin granules. Each chromosome then represents a double twisted chain of chromatin granules, and this double thread twisted on itself, so as to make one end of the chromosome a closed loop and the other with two limbs more or less free.

In the meantime the nucleolus becomes filled with a large number of small vacuolate bodies. Each of these bodies has a dark outer part with a light refractive center. Small bodies exactly like those within the nucleolus appear in the nucleus, and as soon as the nuclear membrane has disappeared some of these are also seen in the surrounding cytoplasm (*figs. 14, 15*). The formation of these micronucleoli occurs as follows: The nucleolus sends out a papilla-like projection, into which one of the vacuolate bodies enters and is then separated from the nucleolus by abstriction (*figs. 16-20*). The micronucleoli are thus all separated from the mother nucleolus by a process of budding. Just about the time when the individual chromosomes are formed and the nuclear membrane disappears, cytoplasmic radiations appear all around the nucleus. These threads pass out

at right angles from the nuclear surface and extend to the walls of the cell. They appear like ordinary cytoplasmic radiations with numerous microsomes (*fig. 15*). Whether these threads are the same as the longitudinal threads of earlier stages I did not determine. However this may be, there is no indication of such crossing threads at this stage. If they were present they should have appeared as well as the divergent ones. It may be that the function of these radiations is to carry the micronucleoli out into the cytoplasm. The micronucleoli are perfectly differentiated by various stains. With anilin-safranin and gentian-violet they have a brilliant red appearance, which makes them stand out more prominently in the sections than they do in the figures. With cyanin and erythrosin the nucleoli are blue, while the surrounding cytoplasm and nucleus are red. They are also differentiated by other stains. The mother nucleoli continue to become smaller, and sometimes some of the chromosomes are collected around the nucleoli in such a manner as to suggest that the nucleoli have something to do with the growth of the chromosomes (*figs. 22a, 23a*). This, however, is doubtless merely an appearance, since twelve chromosomes have not much opportunity to avoid contact with two nucleoli in so small a space. Usually the greater number of chromosomes in a nucleus do not lie in the proximity of the nucleoli at all (*figs. 21-23*). By the time the chromosomes are ready to be arranged at the equator into the mother star the original nucleoli have entirely disappeared, the small daughter nucleoli or micronucleoli being scattered through the surrounding cytoplasm (*figs. 24, 25*). The micronucleoli show a tendency to become placed near the periphery of the cell and away from the nuclear spindle. However, they are often seen quite close to the poles and on the spindle, where with improper staining they might be confounded with the centrospheres. With proper staining, however, there is no possibility of such confusion, for the nucleoli in the cytoplasm have an entirely different structural appearance from the centrospheres, and also show a different staining reaction. There is no doubt in my mind that the micronucleoli

lying on the spindle have often been mistaken for centrosomes, which would explain the instances where many centrospheres have been reported at the ends of the spindle threads; for it is just at this stage that the micronucleoli would have such a position.

The formation of the spindle was not traced. In the mother star stage one centrosome appears very definitely at each pole (*fig. 26*). During metakinesis the centrosome divides into two (*fig. 36 a*). In the daughter skein stage two large centrospheres are sometimes seen at the poles (*fig. 38*). No special effort was made to bring out the centrospheres, and they were not often seen, but wherever they appeared they showed their normal structure and position.

During the formation of the chromosomes from the chromatin band the twisted loops begin to shorten and thicken, giving the appearance of a single twisted linin thread with an irregular double row of chromatin granules. The linin thread also, especially at this stage, stains a very dark purple or black with Delafield's haematoxylin, exactly like the chromatin granules themselves. At this stage also there is a deposit formed around the chromatin loop which gradually becomes thicker as the chromosome reaches maturity (*figs. 21–23 b*). With Delafield's haematoxylin and erythrosin this deposit stains a light pinkish red, while the chromatin band stains a very dark purple. At a later stage, just before the formation of the mother star, the whole chromosome begins to stain deeper, until it finally has a homogeneous appearance when treated with this double stain, and shows no structure whatever, the whole chromosome appearing like a huge mass of chromatin matter (*figs. 24–27*), and it is necessary to employ other stains to differentiate the chromatin band.

When the chromosomes become arranged on the spindle threads in the equatorial plane they are so situated that the end having the two free ends of the chromatin band are attached to the spindle threads, the loop being turned outward and projecting freely beyond the spindle (*figs. 27–28*). There is no

doubt in my mind that this is the case, although it is difficult to determine, but there is a bare possibility that the chromosomes may be turned the other way. However, their position in the nuclear area, and their appearance and behavior on the spindle, indicate that the loops are turned outward. At this stage also the chromosome is generally stained so dark that no trace of the chromatin band is discernible, but in sections stained with anilin safranin, and gentian violet the central part stains darker and clearly indicates the position of the chromatin band (*fig. 30*). The splitting of the chromosome is gradual (*figs. 29–32*), and consists in the separation or untwisting of the chromatin coil, which is gradually pulled out until it lies like a straight band or rope on the spindle threads (*figs. 33–36*). The untwisting of the chromosome can be seen easily in all stages, and by proper focusing the entire coil can be traced. *Figs. 33–35*, which represent the later phases of this process, do not represent the coiled appearance as well as the sections. After the chromatin coils have straightened, the splitting takes place in the middle of each one at the equator. Thus there is an actual transverse division of the chromosomes, the half of each original chromatin loop passing to opposite poles of the spindle. Each daughter nucleus, therefore, receives about as many chromatin granules as there were in the mother nucleus, and although there is no diminution in the number of chromatin granules, only half of the granules originally present in the mother nucleus are represented in each daughter nucleus. It will be seen that although the chromosomes are not all of the same size and length, yet if the chromatin band breaks at practically the middle point each daughter nucleus receives about the same number of chromatin granules; and since the chromatin granules are the same in number as in the mother nucleus it cannot be proper to speak of a reduction in the amount of chromatin, although only half of the original chromatin granules are represented. There is no reduction in number but a reduction of one-half in kind. Whether there is a reduction in the number of chromatin granules before the egg nucleus is formed must

be determined by studying the subsequent divisions. If there is no such subsequent reduction of granules then it would logically follow that the granules must fuse during the union of the sex nuclei. Otherwise the new sporophyte would contain twice the number of chromatin granules that the old one did.

This splitting of the chromosome first longitudinally and then tranversely, it will be seen, really amounts to the same thing as though the original chromosome were divided into four parts, and corresponds to the so-called "tetrad" stage reported by the zoologists. The word tetrad, however, could not properly be applied here, since a true tetrad does not appear.

After the chromosomes have collected about the poles of the spindle and are beginning to form the daughter skeins, cytoplasmic radiations, similar to those seen around the mother nucleus at the time when the micronucleoli were carried out into the cytoplasm, appear, and the micronucleoli often seem to be attached to them (*fig. 37*). Whether these radiations are organized from the centrosomes at the poles, as might seem possible from *fig. 38*, or are the same as those which surrounded the mother nucleus during the migration of the micronucleoli into the cytoplasm, I could not determine. It might be that they remain constantly in the cytoplasm during metakinesis, and only separated somewhat into two parts. As the daughter nuclei become more complete, the micronucleoli collect around them and begin to enter into the nuclei (*figs. 39, 40*). As they enter the nuclei and fewer are left in the surrounding cytoplasm, the cytoplasmic radiations become less distinct, and they finally disappear altogether when the nucleoli have all entered the daughter nuclei (*figs. 40–43*). The micronucleoli as they enter into the nuclei build up new daughter nucleoli by a continuous process of aggregation and fusion (*figs. 40–43*).

During the divisions of the two daughter nuclei which produce the four-celled embryo sac, the nucleoli act in exactly the same way as has been described for the first division, and the same kind of cytoplasmic radiations arise (*figs. 44–46*). In the divisions which complete the embryo sac, the same process was

observed to occur; so it can be stated without exception that this action of the nucleoli in being thrown out into the cytoplasm and collected again into the daughter nuclei is the normal process for the whole gametophyte generation of *L. Philadelphicum*. Whether this process will be found to occur in the gametophyte generation of all angiosperms, or in all plant cells, is yet to be determined. There is often a marked peripheral placing of the nucleoli in the daughter nuclei which becomes very striking in certain cases where the nuclei lie in just the proper position (*fig. 47*). This would in itself be quite suggestive of the way in which the nucleoli were formed, even if they were not seen to enter from the outside.

The micronucleoli are constantly present in the cytoplasm from the time they leave the nucleus until they enter again. Of course it may be urged that the original micronucleoli are dissolved in the cytoplasm and new ones formed. If this is the case the dissolution of old ones and the formation of new ones must go on simultaneously. It is not intended to contend here that the nucleolus is a permanent cell organ, for more observation is needed for such a generalization. But that the nucleoli pass out and enter again to form new ones in the daughter nuclei cannot be denied. The strongest argument in favor of regarding the nucleolus as a definite body or organ seems to the writer to be the fact that in many plants and tissues the number is constant. Thus in many cases the number in each nucleus is almost absolutely constant. Are such examples of constancy at hand for other excretions or food products? That the number is often variable is no argument against its fundamental character. The number of nuclei in many cells is also exceedingly variable.

During the divisions of the nuclei in the embryo sac the spindle threads undergo a thickening in the middle as though a nuclear plate and cell-wall were to be formed (*figs. 38–41*), and the spindle often persists from one division to another, so that four daughter nuclei may appear to be connected by three spindles (*figs. 45, 46*). This thickening of the spindle threads

would seem to be an inheritance from a true thallus with regular cell walls.

GENERAL DISCUSSION.

Chromosomes.—It has been claimed by botanists, especially Guignard and Strasburger, that during the karyokinetic division of the reduction nucleus in plants the chromosomes undergo longitudinal division just as in ordinary vegetative cells. This has also been maintained by Boveri, Hertwig, and Brauer in regard to Ascaris, where the so-called "tetrad" is said to arise by a double longitudinal splitting of the primary chromatin rod. Recently, however, it has been found by Rückert, Häcker, and vom Rath, that in certain arthropods each "tetrad" arises by one longitudinal and one transverse division of the primary chromosome. This would make a true reduction in Weismann's sense.

Since writing the present investigation the author has read a paper by Calkins[1] in which the formation of "tetrad" chromosomes is described as occurring in the spore mother cells of two ferns, *Pteris tremula* and *Adiantum cuneatum*. The author is quite certain that transverse division occurs in these chromosomes, although he could not tell whether the reduction took place in the first division or in the division following. He thinks, however, that the first division is longitudinal and the second one transverse, so that the reduction would take place in the second division. This, however, is merely an inference, and he seems to have no direct evidence as to when the transverse division takes place, if it occurs at all. Although the work is a very commendable one the author's substitution of zoological for botanical terms seems unwise, since it is still doubtful whether the zoologists have arrived at the exact truth in every case or not. The term "tetrad" in connection with the chromosomes is especially objectionable in botany, since "tetrad" had a definite meaning many years before chromosome "tetrads" were

[1] Chromatin reduction and tetrad-formation in pteridophytes. Bull. Torr. Bot. Club **24**: 101–115. 1897.

thought of. A different term seems advisable in order to avoid the confusion which must arise if it should be introduced into botany.

Mottier[2] has also reported a transverse division of the chromosomes in the pollen mother cells of Lilium. He reports that the pseudo-reduction takes place in the first division and the transverse splitting of the chromosomes in the second. However, his evidence is not very conclusive, and his figures are rather indefinite, so that it is not possible to judge whether his conclusions are justifiable or not. In the case of the reduction nucleus of the embryo sac of *L. Philadelphicum* the divisions which form the macrospores are skipped, so I am not able to generalize or predict what would occur in the normal process where a number of macrospores or microspores are formed.

If we accept Dixon's evidence, it seems probable that the reduction takes place in the first division of the pollen mother cells. In the pollen mother cells of *L. longiflorum*, Dixon[3] found that during the first division the chromosomes sometimes formed a loop which he thinks may be derived possibly from a loop in the original chromatin band, and sometimes they are twisted round each other. He says that while they lie in the equator the two parts of the chromosome are in close contact and seem fused together at their inner extremities, and that during metakinesis the two rod-like portions part from one another. He says: "From the process described it appears probable that each chromosome in this karyokinesis represents two of the previous nuclear divisions which have become more or less completely united end to end." "Thus the reduction in number is effected by an end to end fusion of the chromosomes as Strasburger has already suggested." "The next division by which the pollen tetrads are formed takes place probably according to the normal karyokinesis in plant-cells."

It must be borne in mind, however, that there is at present

[2] Beiträge zur Kenntniss der Kerntheilung in den Pollenmutterzellen einiger Dikotylen und Monokotylen, Jahrb. f. wiss. Bot. **30**: 169–204. 1897.

[3] The nuclei of Lilium longiflorum. Ann. Bot. **9**: 663–665. 1895.

no possible way of telling in such forms as Lilium what becomes of the individual chromosomes which go to make up the spirem of the reduction nucleus. The spirem is a continuous thread before it breaks up, and to say that each piece represents two of the former chromosomes is a mere assumption dangerous to make. It is just as probable, so far as we know, that the breaking may take place anywhere, and that the individuality of the former chromosomes is entirely lost.

Turning now to Guignard's [4] figures of the division of the pollen mother cells and embryo sac nucleus of *L. Martagon*, we find a wonderful agreement in his figures with my own. If the chromosomes represented in Guignard's *figs. 48* and *49* had the chromatin band closed into a loop they would agree exactly with mine from this stage on. His figures indicate that the process of splitting was the same as I have determined, but that he was misled by thinking that his double chromatin band was open at both ends. And were no conflicting evidence at hand I should say that his figures of the divisions in the pollen mother cells establish a transverse splitting of the chromosomes for the first division of the pollen mother cells and not for the second. Whatever the fact may be, it is to be hoped that evidence on this point will soon be offered which will relieve the present uncertainty.

It will be seen, as already stated, that in *L. Philadelphicum*, because of longitudinal splitting of the spirem and its subsequent transverse division during metakinesis, we have to do with exactly similar phenomena as those which have been described for "tetrad" formation, although the chromosome never gives any appearance of a separation into four distinct parts, but as has been described forms a double twisted coil. The early longitudinal division of the spirem, before it breaks up into the reduced number of chromosomes, has also been found by vom Rath [5] in the insect *Gryllotalpa vulgaris*.

[4] Nouvelles Études, etc. Ann. Sci. Nat. Bot. VII. **14**: 163–296. 1891.

[5] Zur Kenntniss der Spermatogenese von Gryllotalpa vulgaris. Arch. f. Mik. Anat. **40**: —. 1892.

Miss Sargent[6] has recently studied the division of the reduction nucleus of *L. Martagon*, but was unable to detect any transverse division of the chromosome, although some facts observed by her point that way. She has much to say in regard to synapsis, but I am fully convinced that many of the appearances she describes were due to poor treatment of the material. In fact, I regard the so-called stage of synapsis as simply a product of poor preparation. In none of my better preparations have I found such an appearance, in fact it was so rare in the stages where it is reported to occur that I should have missed it altogether had I not made a careful search for its appearance, although I had a large number of my own preparations and had the privilege of looking over a large number put up by others in the laboratory. In the stage when the contraction usually occurs the chromatin band is quite free within the nuclear membrane, since it is at this time twisting and orienting itself to form the twelve chromatin loops. Everything therefore is favorable for an artificial contraction. When the contraction does take place it is often exactly in the middle of the nuclear area, and sometimes it occurs in such a manner that the large nucleolus is left entirely free in the nuclear area away from the mass of chromatin. Miss Sargent's explanation, therefore, that the chromatin contracts around the nucleolus in order to keep this "washy" looking body from escaping beyond the confines of the chromatin because of its supposed dissolution at this period, I venture to regard as erroneous. In various other plants I have seen contractions of this sort, but always in very poor preparations, from which it would not be wise to draw conclusions, and I believe that I am safe in saying that the nucleolus is much more often free in the nuclear area than caught in the contracted meshes of the spirem.

Although a transverse division of the chromosome, or a true reducing division, is here established for the macrospore of *L Philadelphicum*, the writer does not wish to be understood as

[6] The formation of the sexual nuclei in Lilium Martagon. I. Oogenesis. Ann. Bot. **10**: 445–477. 1896.

thinking that this will give any support to Weismann's theory of heredity. It has been shown already by certain botanists that this theory breaks down entirely in the case of plants. Whether Strasburger's theory of reduction is correct or not the future must decide. We are evidently not yet ready to establish a theory of reduction, as we are dealing with phenomena concerning which hardly two observers agree.

Nucleoli.—Apparently no structure in the cell is so little known as the nucleolus, and one needs only examine the literature of the subject to discover how little has been done that can stand the test of criticism. One of the most common ideas in regard to this body was that it represents a globule of food material which can be used by the nucleus or cell in general as occasion demands. Strasburger,[7] in 1895, suggested that the substance of the nucleoli serves for the construction of the spindle threads, but it is doubtful whether there ever was any very strong evidence in favor of such a view. Went,[8] in 1887, saw in the endosperm nuclei that the nucleoli were lying in contact with the chromatin threads, and he thought the substance of the nucleoli was taken up by the chromosomes. It is very doubtful, however, whether any part of the nucleoli ever goes to aid in the formation of the chromosomes. Tangl[9] was the first to observe the extrusion of the nucleolus into the cytoplasm. This was seen in the pollen mother cells of *Hemerocallis fulva*. Karsten[10] attempted to show that the centrosomes had their origin in extruded nucleoli. It was soon shown by Guignard and others, however, that this was not the case, and that Karsten did not see true centrosomes at all. Belajeff[11] has the nucleoli passing out into the cytoplasm, but he claims that they are dissolved and that new ones arise in the daughter nuclei.

[7] Karyokinetische Probleme. Jahrb. f. wiss. Bot. **28**: 151–204. 1895.

[8] Beobachtungen über Kern- und Zelltheilung. Ber. d. d. bot. Ges. **5**: 247. 1887.

[9] Die Kern- und Zelltheilung bei der Bildung des Pollens von *Hemerocallis fulva* L. Denkschr. d. Kais. Akad. d. Wiss. zu Wien. **45**: 65. 1882.

[10] Ueber Beziehungen der Nucleolen zu den Centrosomen bei *Psilotum triquetrum*. Ber. d. d. bot. Ges. **11**: 555–562. 1893.

[11] Zur Kenntniss der Karyokinese bei den Pflanzen. Flora —: 436. 1894.

A. Zimmermann[12] found that at the beginning of nuclear division the nucleoli pass out of the nucleus into the cytoplasm, and that later in the metaphase of the division they are again taken up into the daughter nucleus. He advances the proposition "omnis nucleolus e nucleolo." This process was observed in the pollen mother cells of *Lilium Martagon*, where the nucleoli are said to break up into numerous small granules. Zimmermann also observed the extrusion of the nucleoli into the cytoplasm in *Hyacinthus candicans*, *Fritillaria imperialis*, *Equisetum*, and *Psilotum*. The extrusion was also found in the primary embryo sac nucleus of *Lilium Martagon* and *Fritillaria imperialis*, in the vegetative cells of the root tips of *Vicia faba*, and in the stem tips of *Phaseolus communis* and *Psilotum triquetrum*. Rosen[13] has also found nucleoli in the cytoplasm of the cells of roots of Hyacinthus. Zimmermann's observations were immediately disputed by Guignard, Humphrey, and Strasburger. Humphrey[14] considers that the phenomena recorded by Zimmerman are the results of manipulation.

Zimmermann believes that the nucleoli which are thrust out into the cytoplasm return again into the daughter nuclei. In his last work[15] he still thinks that this occurs in some cases, but that it may not be universal. He considers that there is a fusion of the nuclei even in the cytoplasm, and I have also found that this seems to be the case, although generally no fusion of micronucleoli occurs until they have entered the daughter nucleoli. Whether the nucleoli are real organs of the cell of course still remains an open question, but for the divisions that take place in the embryo sac of *Lilium Philadelphicum*, my own study assures me that in the prophase of nuclear division the nucleoli give rise to a large number of micronucleoli which are carried out into the cytoplasm, and in the metaphase of the

[12] Ueber das Verhalten der Nukleolen während der Karyokinese. Beiträge zur Morphologie und Physiologie der Pflanzenzelle **2**: 1–35. 1893. Tübingen.

[13] Beiträge zur Kenntniss der Pflanzenzellen. Beitr. z. Biol. der Pflanzen (Cohn). **7**: 225–312. 1895. [Heft 2].

[14] Nucleolen und Centrosomen. Ber. d. d. bot. Ges. **12**: 108–117. 1894.

[15] Die Morphologie und Physiologie des pflanzlichen Zellkernes. Jena. 1896.

division they are again collected into the daughter nuclei, where by repeated fusions they form the large nucleoli of the mature daughter nuclei.

Cytoplasmic radiations and spindle threads.—The tangential threads which I observed in the early stages of the development of the macrospore nucleus are no doubt the same as those recently described by Mottier and others as being the beginnings of the achromatic spindle. They do not converge to definite points, however, but pass almost in straight lines across the cell from one wall to the other, the greater number appearing to pass in a direction longitudinal to the long axis of the growing macrospore. From their varying direction it follows that there must be numerous points of intersection, but it does not appear that any number intersect at a given point. At a later stage, in very fine sections, these radiations do not appear at all, but a new set of cytoplasmic threads appear diverging in every direction, and at right angles to the nuclear surface. These radiations are the same as those figured so beautifully by Guignard. Whether these diverging radiations represent the same structures as the earlier tangential threads or not I could not determine. They were never seen to converge to definite points, but I can easily imagine how in a contracted condition of the cytoplasm they might give such an appearance. My belief is that the real purpose of these radiations is to carry out the micronucleoli into the cytoplasm. They appear just when the micronucleoli begin to migrate, and it is difficult to imagine how the nucleoli could pass outward unless carried by streams of cytoplasm.

The formation of the spindle was not followed, but in the mother star stage the spindle is always bipolar and ends in a definite point. No appearance of a multipolar spindle was ever observed, unless in cases where the spindle had been cut or torn Even granting that the spindle is formed as Farmer states, there may still be two unseen centrospheres toward which all the small poles of the multipolar spindles are attracted. It is inconceivable to the writer how a definite bipolar spindle should

always be formed from several minor poles which extend in every direction, without the control of some body or other influence at a definite point. The writer has preparations in which very definite centrospheres are to be seen at the poles of the mother star. These preparations have been examined by a large number of experienced observers at more than one laboratory, so that the sweeping assertion that no such bodies have ever been observed in the higher plants is certainly unwarranted.

In this connection it might also be proper to speak of the large spherical bodies figured by Farmer[16] on his multipolar spindles in the pollen mother cells of *Lilium Martagon*. There is no doubt in my mind, from the description and figures, that these were micronucleoli, and had nothing whatsoever to do with the spindle, especially as he speaks of granules which are colored by those stains which differentiate the chromatic elements of the nucleus.

It must be remembered that it is an impossibility to find the spindle converging to a single pole when the nucleus and spindle have been cut into half a dozen slices, and more often cut diagonally than longitudinally. No one denies that multipolar spindles are to be seen in such cases. But it will be remembered that most of these so-called multipolar spindles have been reported in just such cases, nearly always in connection with the enormous nuclei of the pollen mother cell or the reduction nucleus of the embryo sac. The cell and its contents would have to be made of steel or flint if it were to preserve its centrospheres and poles in proper position after having been cut into half a dozen or more sections. It is even claimed that in the thinnest sections it is easily seen that the spindle does not come to a pole, which is certainly not surprising.

The numerous radiations which appear around the daughter nuclei, if the suggestion is correct for those around the mother nucleus, are for the purpose of returning the micronucleoli into the daughter nuclei. As has been said, the micronucleoli are

[16] On nuclear division in the pollen mother cells of *Lilium Martagon*. Ann. Bot. 7: 392–396. 1893.

often seen on these radiations on their return, and the radiations disappear as soon as all the micronucleoli have entered the daughter nuclei, as if there remained no further function for them. There is no spindle to be formed with which they might be associated, as is the case with those around the mother nucleus.

That the central spindle threads are not necessary for the formation of the daughter nuclei is shown by the fact that the entire central spindle may persist in the cytoplasm for several divisions. At some future time the writer hopes to discuss the origin of the spindle threads and their relations to the centrosomes.

SUMMARY.

1. In *Lilium Philadelphicum* the archesporial cell develops directly into the macrospore, and its nucleus during the first-division appears with twelve chromosomes, or half the number which are present in the vegetative nuclei. At quite an early stage in the development of the macrospore the linin thread of the chromatin network begins to thicken, and the chromatin granules undergo transverse fission. After division of the chromatin granules the whole chromatin band undergoes longitudinal splitting, and the double threads thus produced begin to twist upon each other. This twisted band finally manifests itself as a single continuous spirem, which doubles up and twists into twelve loops. The twelve loops break apart and give rise to the twelve chromosomes. The two linin threads with their granules, which compose the loop, continually become more intimately associated, so that the loop appears like a single linin thread with two irregular rows of chromatin granules. The chromatin loops become shorter by contraction, and receive a thick deposit of some substance which stains light at first but later takes the same color as the chromatin, giving the chromosomes the appearance of homogeneous, somewhat irregularly bean shaped bodies, in which the original chromatin loop is rarely visible The chromosomes arrange themselves in

the equatorial plane in such a manner that the end containing the two free ends of the original chromatin loop is in contact with the spindle fibers. The fibers gradually pull the chromosomes apart in such a manner that the original chromatin loop is untwisted and finally cut in two by a transverse division. Thus the parts of each chromosome which pass to the daughter nuclei represent transverse halves of the original chromatin loop, formed from about one twelfth of the double spirem.

2. The nucleus at first usually has about three nucleoli, each with one or more large granular vacuoles. After the longitudinal splitting of the chromatin band there arise in the nuclei numerous small vacuolate bodies. These are successively abstricted from the mother nucleolus by a process of budding, and give rise to numerous micronucleoli, which all pass out into the cytoplasm before the formation of the mother star, and later, at about the beginning of the close daughter skeins, these micronucleoli all pass back into the daughter nuclei, and by aggregation form the new nucleoli of the daughter nuclei. This process is repeated for every division of the female gametophyte.

3. At about the time of the division of the chromatin granules, there appear in the cytoplasm peculiar cytoplasmic threads, which pass from one side of the cell to the other, and are mostly tangent to the nucleus. At a later stage, at about the beginning of the nucleolar migration, these threads have disappeared, and numerous radiating threads pass out at right angles from the nuclear surface and extend to the cell walls. These radiations seem to hold some relation to the migration of the micronucleoli. Similar radiations appear around the daughter nuclei, and the micronucleoli, as they are drawn into the daughter nuclei, seem to be in contact with these cytoplasmic threads.

4. Two centrospheres appear beside the resting nucleus, and in the mother star stage a single centrosphere appears at each pole of the spindle; while a little later, during metakinesis, a centrosphere appears at each point with a double centrosome.

In the daughter skein stage there are two centrospheres at each pole, which are often quite distinct and can easily be differentiated from the micronucleoli.

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EXPLANATION OF PLATES XXXVII–XXXIX.

All the figures are reduced to three-eighths of their original size. The magnification given with each figure is the original magnification of the drawings before reduction.

FIG. 1. Part of a young nucellus showing the archesporium developing directly into a fertile macrospore. Anilin-safranin, gentian-violet. × 1200.

FIG. 1*a*. A small part of the chromatin network showing a single row of chromatin granules on the linin threads. × 2250.

FIG. 2. Nucleus a little older than in *fig. 1*. The chromatin network seems to be in close connection with the nucleoli. Chromatin granules considerably larger. Anilin-safranin, gentian-violet. × 1200.

FIG. 2*a*. Linin thread with chromatin granules.

FIG. 3. Nucleolus; the usual appearance before the division of the chromatin granules. Delafield's haematoxylin, erythrosin. × 2250.

FIG. 4. Section of nucleus at the stage when the integuments are just beginning to appear. The linin thread is very thick and the chromatin granules are dividing transversely. There were four large nucleoli in this nucleus. Delafield's haematoxylin, erythrosin. × 1200.

FIG. 4*a*. Small piece of the linin thread showing arrangement of the chromatin granules. × 2250.

FIG. 5. An enormous nucleolus, with large central vacuole, which has a granular structure. Just before division of the chromatin granules. Anilin-safranin, gentian-violet. × 2250.

FIG. 6. Nucleolus about the same stage as *fig. 5*, with a depression or dent on one side. Anilin-safranin, gentian-violet. × 2250.

FIG. 7. Nucleolus with several vacuoles; same stage as *figs. 6* and *7*. Anilin-safranin, gentian-violet. × 2250.

FIG. 8. Macrospore with nucleus containing double chromatin bands produced by longitudinal splitting. Two centrospheres on one side of the nucleus. The cytoplasm contains peculiar radiating strands. Delafield's haematoxylin, erythrosin. × 1200.

FIG. 8*a*. Section of the same nucleus showing another nucleolus. × 1200.

FIG. 8*b*. Short piece of double chromatin band. × 2250.

FIG. 8*c*. Double chromatin band a little wider than *fig. 8b*. × 2250.

FIG. 9. Thin section of a macrospore with nucleus containing double rows of chromatin bands. The nucleolus shows a number of small vacuoles. Anilin-safranin, gentian-violet. × 1200.

FIG. 9*a*. A single thread of the double chromatin band showing the arrangement of the chromatin granules on the linin thread. × 2250.

FIG. 10. Nucleus in which the double threads of the chromatin band are beginning to twist on each other. Anilin-safranin. gentian-violet. × 1200.

FIG. 10*a*. A double twisted chromatin band crossed by a single one. The other part was very likely cut away. × 2250.

FIG. 11. Section of a nucleus in which the double twisted chromatin band has twisted into twelve loops with the heads toward the nuclear membrane. Anilin-safranin, gentian-violet. × 1200.

FIG. 11*a*. Adjoining section of the same nucleus. × 1200.

FIG. 11*b*. Two loops from *fig. 11*. × 2250.

FIG. 12. Chromatin loop a little later than *fig. 11*. The two linin threads have twisted more closely together, presenting more nearly the appearance of a single band. Anilin-safranin, gentian-violet. × 2250.

FIG. 13. Thin section of macrospore showing chromosomes immediately after the breaking up of the chromatin band into the twelve chromosomes. The nuclear membrane has almost disappeared. Delafield's haematoxylin, erythrosin. × 1200.

FIG. 14. Section of macrospore showing the double nature of the chromatin loops, a large vacuolate nucleolus, and three micronucleoli, one of which has passed beyond the nuclear limits. Anilin-safranin, gentian-violet. × 1200.

FIG. 15. Section of macrospore showing fine cytoplasmic radiations extending outward from the nucleus. In the nuclear area is one large nucleolus and numerous micronucleoli. Some of the micronucleoli have traveled far out into the cytoplasm. Anilin-safranin, genatin-violet. × 1200.

FIG. 16. Nucleolus with vacuolate bodies; just before the breaking up of the chromatin band. Anilin-safranin, gentian-violet. ×2250.

FIG. 17. Nucleolus with vacuolate bodies. Anilin-safranin, gentian-violet. × 2250.

FIG. 18. Nucleolus with small spherical vacuolate bodies. A small nucleolus lying outside of the nuclear boundary. Anilin-safranin, gentian-violet. × 2250.

FIG. 19. Nucleolus with one of the vacuolate bodies apparently being extruded. Anilin-safranin, gentian-violet. ×2250.

FIG. 20. Outline of a nucleus, showing one large vacuolate nucleolus and a micronucleolus within the nuclear area, a small vacuolate nucleolus outside. Anilin-safranin, gentian-violet. × 2250.